Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for determining presence or absence of oxidative reactions catalyzed by at least one enzyme in a solution comprising:

(i) contacting said solution with a sensor composition which comprises a luminescent compound that exhibits a change in a luminescent property thereof, when irradiated with light containing wavelengths which cause said luminescent compound to luminesce, upon exposure to oxygen, wherein the presence of said ~~the~~ sensor composition is non destructive to said at least one enzyme;

(ii) irradiating said sensor composition with a light containing wavelengths which cause said luminescent compound to luminesce;

(iii) measuring or visually observing said ~~the~~ luminescent property of ~~from~~ said luminescent compound while irradiating said sensor composition with said light;

(iv) comparing said luminescent property of said luminescent compound ~~measurement~~ to ~~that~~ a luminescent property of a control, wherein said control is selected from the group consisting of:

a reagent control not containing at least one enzyme capable of catalyzing said oxidative reactions and a calculated threshold; wherein a change in said luminescent property of said

luminescent compound relative to said luminescent property of said ~~the~~ control is indicative of the presence or absence of said oxidative reactions; and

(v) in the event that no change in said luminescent property of said luminescent compound relative to said luminescent property of said ~~the~~ control is measured or observed, repeat steps (ii), (iii), (iv) as needed, to determine the presence or absence of said oxidative reactions in said solution.

Claim 2 (original): The method of Claim 1 wherein said luminescent compound is contained within a matrix which is relatively impermeable to water and non-gaseous solutes, but which has a high permeability to oxygen.

Claim 3 (original): The method of Claim 2 wherein said matrix is a rubber or plastic matrix.

Claim 4 (original): The method of Claim 2 wherein said matrix is a silicone rubber matrix.

Claim 5 (original): The method of Claim 2 wherein said luminescent compound is adsorbed on solid silica particles.

Claim 6 (original): The method of Claim 1 wherein said luminescent compound is a tris-4, 7-diphenyl-1, 10-phenanthroline ruthenium (II) salt.

Claim 7 (original): The method of Claim 6 wherein said luminescent compound is tris-4, 7-diphenyl-1, 10-phenanthroline ruthenium (II) chloride.

Claim 8 (original): The method of Claim 1 wherein said luminescent compound is a tris-2, 2'-bipyridyl ruthenium (II) salt.

Claim 9 (original): The method of Claim 8 wherein said luminescent compound is tris-2, 2'-bipyridyl ruthenium (II) chloride hexahydrate.

Claim 10 (original): The method of Claim 1 wherein said luminescent compound is 9, 10-diphenyl anthracene.

Claim 11 (original): The method of Claim 1 wherein said solution is isolated from atmospheric oxygen wherein said solution is contained in a closed system.

Claim 12 (original): The method of Claim 1 wherein said solution is exposed to atmospheric oxygen.

Claim 13 (canceled)

Claim 14 (original): The method of Claim 1 where the oxidative reactions are performed by metabolic enzymes in liver cells or cells which have been modified to express metabolic enzymes.

Claim 15 (original): The method of Claim 1 where the oxidative reactions are performed by several enzymes which together comprise a subcellular system.

Claim 16 (original): The method of Claim 15 where the enzymes include a cytochrome P450 and a P450 reductase.

Claims 17-48 (canceled)

Claim 49 (currently amended): A method for determining presence or absence of oxidative reactions catalyzed by at least one enzyme in a solution comprising:

(i) placing said solution in a container in which said solution ~~fluid~~ is substantially isolated from atmospheric oxygen and placing within said container, but not in direct contact with said solution ~~fluid~~, a sensor composition which comprises a luminescent compound that exhibits a change in a luminescent property thereof, when irradiated with light containing wavelengths which cause said luminescent compound to luminesce, upon exposure to oxygen, wherein the presence of said ~~the~~ sensor composition is non-destructive to said at least one enzyme;

(ii) irradiating said sensor composition with a light containing wavelengths which cause said luminescent compound to luminesce;

(iii) measuring or visually observing said ~~the~~ luminescent property of ~~from~~ said luminescent compound while irradiating said sensor composition with said light;

(iv) comparing said luminescent property of said luminescent compound ~~measurement~~ to ~~that~~ a luminescent property of a control, wherein said control is selected from the group consisting of:

a reagent not containing at least one enzyme capable of catalyzing said oxidative reactions and a calculated threshold, wherein a change in said luminescent property of said

luminescent compound relative to said luminescent property of said ~~the~~ control is indicative of the presence or absence of said oxidative reactions; and

(v) in the event that no change in said luminescent property of said luminescent compound relative to said luminescent property of said ~~the~~ control is measured or observed, repeat steps (ii), (iii), (iv) as needed, to determine the presence or absence of oxidative reactions in said solution.

Claim 50 (original): The method of Claim 49 wherein said luminescent compound is contained within a matrix which is relatively impermeable to water and non-gaseous solutes, but which has a high permeability to oxygen.

Claim 51 (original): The method of Claim 50 wherein said matrix is a rubber or plastic matrix.

Claim 52 (original): The method of Claim 50 wherein said matrix is a silicone rubber matrix.

Claim 53 (original): The method of Claim 50 wherein said luminescent compound is adsorbed on solid silica particles.

Claim 54 (original): The method of Claim 49 wherein said luminescent compound is a tris-4, 7-diphenyl-1, 10-phenanthroline ruthenium (II) salt.

Claim 55 (original): The method of Claim 54 wherein said luminescent compound is tris-4, 7-diphenyl-1, 10-phenanthroline ruthenium (II) chloride.

Claim 56 (original): The method of Claim 49 wherein said luminescent compound is a tris-2, 2'-bipyridyl ruthenium (II) salt.

Claim 57 (original): The method of Claim 56 wherein said luminescent compound is tris-2, 2'-bipyridyl ruthenium (II) chloride hexahydrate.

Claim 58 (original): The method of Claim 49 wherein said luminescent compound is 9, 10-diphenyl anthracene.

Claim 59 (original): The method of Claim 49 wherein, in step (i), the solution is also contacted with an effective concentration of one or more biomaterials.

Claim 60 (original): The method of Claim 49 where the oxidative reactions are performed by metabolic enzymes in liver cells or cells which have been modified to express metabolic enzymes.

Claim 61 (original): The method of Claim 49 where the oxidative reactions are performed by several enzymes which together comprise a subcellular system.

Claim 62 (original): The method of Claim 61 where the enzymes include a cytochrome P450 and a P450 reductase.

Claim 63-90 (canceled)

Claim 91 (previously presented): The method of claim 1 wherein said solution is further contacted with liquid or semi-solid biomaterials for promoting or enabling cellular growth and respiration.

Claim 92 (canceled)

Claim 93 (currently amended): The method of claim 91 ~~92~~ wherein said biomaterial is an extracellular matrix ~~Matrigel~~.

Claim 94 (previously presented): The method of claim 1 wherein, in step (i) said solution also contacted with an effective concentration of one or more extracellular matrices, said effective concentration being effective for promoting or enabling cellular growth and respiration.

Claim 95 (previously presented): The method of claim 94 wherein said extracellular matrix is collagen.

Claim 96 (currently amended): The method of claim 1 wherein, in step (i), said solution is contacted with an effective concentration or one or more additives or coating substances, selected from the group consisting of penicillin, streptomycin, fungizole ~~hingizone~~, non-essential amino acids, sodium pyruvate, and fetal bovine serum, said effective concentration being effective for promoting or enabling cellular growth and respiration.

Claim 97 (previously presented): The method of claim 49 wherein said solution is further contacted with liquid or semi-solid biomaterials for promoting or enabling cellular growth and respiration.

Claim 98 (canceled)

Claim 99 (currently amended): The method of claim 97 ~~98~~ wherein said biomaterial is an extracellular matrix ~~Matrigel~~.

Claim 100 (currently amended): The method of claim 49 wherein, in step (i) said solution also contacted with an effective concentration of one or more extracellular matrices, said effective concentration being effective for promoting or enabling cellular growth and respiration.

Claim 101 (previously presented): The method of claim 100 wherein said extracellular matrix is collagen.

Claim 102 (currently amended): The method of claim 49 wherein, in step (i), said solution is contacted with an effective concentration or one or more additives or coating substances, selected from the group consisting of penicillin, streptomycin, fungizone ~~hingizone~~, non-essential amino acids, sodium pyruvate, and fetal bovine serum, said effective concentration being effective for promoting or enabling cellular growth and respiration.

REMARKS/ARGUMENTS

The status of the claims in the application to date is as follows: 1-12, 14-16, 49-62, and 91-102 are in the application. Claims 1-12, 14-16, 49-62, and 91-102 have been rejected. Claims 92 and 98 are canceled herein. Claims 93, 94, 96, 99, 100 and 102 are amended herein. No new matter has been added.

The Examiner has requested the status of the related applications be updated in the specification. The cross reference data has been entered by this amendment obviating the grounds for the objection.

In addition, the Examiner has requested the Applicants inform the Examiner as to how this application differs from each of the parent applications so the proper priority date may be granted. The request for this delineation is respectfully traversed in view of the present amendment. The lineage of the parent case is now set forth. The Examiner's attention is called to the fact that certain of these cases are continuation-in-part applications. These continuation-in-part applications claim common priority for common subject matter as is permitted by the rules of practice. In absence of a specific rejection directed to certain subject matter, undersigned counsel is unaware of any requirement to make the delineation requested by the Examiner. Reconsideration is respectfully requested.

Response to Rejection of Claims Under 35 U.S.C. 112, first paragraph

The Examiner has rejected claims 92, 96, 98 and 102 as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor had possession of the claimed invention. The Examiner further alleges that Table 12 does not disclose the materials listed therein. Claim 92

and 98 have been canceled obviating the grounds of rejection. Applicants respectfully traverse the rejection with respect to the remaining claims.

Applicants respectfully submit that the claims are sufficiently supported in the specification. Regarding claim 91, biomaterials are discussed generally, for example, on page 24, lines 14-16 of the specification. Regarding claims 96 and 102, additives or coatings are disclosed in numerous locations throughout the specification. Penicillin and streptomycin are disclosed in Example 18 on page 43; fungizole is disclosed in Example on page 42, line 25; non-essential amino acids are disclosed on page 48 in Table 12 and in the examples, such as Example 29 on page 50; and fetal calf serum, also referred to as fetal bovine serum or FBS, is disclosed in the examples, such as the materials and methods on page 42, Example 18 on page 43, Example 29 on page 50 and Example 39 on page 57. Additionally, sodium pyruvate is disclosed on page 48 in Table 12. It is clear from a thorough reading of the specification as a whole, which includes Table 12, that the components described as supplements in the table are understood as additives or supplements to sustain and/or promote growth. Applicants respectfully submit that claims 91, 96 and 102 contain subject matter which is sufficiently described in the specification to establish possession of the claimed invention. Withdrawal of the rejection of claims 92, 98 and 102 is respectfully requested in light of the above.

Response to Rejection of Claims Under 35 U.S.C. 112, second paragraph

The Examiner has rejected claims 91 to 102 under 35 U.S.C. 112, second paragraph as allegedly failing to particularly point out and distinctly claim the subject matter the inventors regard as the invention. Furthermore, the Examiner has rejected the title of the invention as not aptly descriptive. The claims and title have been amended to address the rejections. These changes were not made for the purposes of defining or otherwise limiting the meaning of a term

or for prior art purposes. Applicant respectfully requests reconsideration of the rejection of claims 91-102 and the title under 35 U.S.C. §112, second paragraph, in light of the above.

Response to Rejection of Claims Under 35 U.S.C. §103

The Examiner has rejected claims 1-12, 14-16, 49-62 and 91-102 under 35 U.S.C. §103 as allegedly being unpatentable over each of the article by Bacon and the article by Parker. Specifically, the Examiner alleges that the Bacon reference teaches immobilization of certain ruthenium dyes in a silicone rubber for measuring oxygen concentrations. The Examiner alleges that the Parker article teaches use of fluorescence quenching of an anthracene dye to measure oxygen concentration which can be immobilized in a polymer such as silicone. The reactions may be performed in cuvettes.

The Examiner admits that the independent claims differ from the above references in that they recite the enzyme is in solution. Further, the dependent claims 5, 53, 8, 9, 56, 57, 14-16 and 60-62 differ from the references, yet concludes that it would have been obvious to one of ordinary skill in the art to modify the references to include these differences. The Examiner also states that no novelty is seen in employing repeated measurements as needed because no need is shown and no precision is required. Applicants respectfully traverse the rejection.

Before responding to the rejections, Applicants would like to summarize the invention. The present invention provides methods of identifying the presence or absence of oxidative reactions, such as those catalyzed by enzymatic activity, by contacting a sample or air above the sample with a sensor. The sensor includes a luminescent compound that is protected from destructive activity of the sample. The luminescent compound experiences a change in luminescent property which corresponds to a change in oxygen concentration. The change in

oxygen concentration is proportional to the amount and/or rate of oxidative reduction reactions. The protection of the luminescent compound allows for detection of, for example, oxidative reactions occurring as a result of cells metabolizing a drug candidate, without destroying the sensor. It is possible to use the sensor in subsequent analyses because the sensor is not destroyed by the sample.

Applicants respectfully traverse the rejection raised by the Examiner. Applicants respectfully disagree with the Examiner's statement of the teachings of Parker and Bacon. As discussed in detail below, the prior art, in combination with the knowledge generally available in the art at the time of the invention, does not provide sufficient motivation to modify the references. Additionally, the prior art fails to teach or suggest all the limitations of the claims. As a result, a *prima facie* case of obviousness has not been established.

In Bacon, problems of using luminescent compounds in detecting oxygen are discussed which include interference from gaseous or solution interferents. See page 2781, column 1. One solution to the problem, as discussed in the reference, is to isolate the dye by covalently attaching it to a material or to microencapsulate the dyes. The isolation is understood as useful to minimize leaching effects and to isolate the dye from the environment.

Another problem identified in Bacon, is that "In practice, an excited state (of a dye) that is susceptible to quenching by one species is generally susceptible to quenching by a variety of materials, and the number of interferents is large enough to make solution luminescence quenching nearly unusable." See page 2781, column 1, sixth full paragraph. The answer to the problem, as presented by Bacon, is to immobilize the oxygen sensor in a solvent impermeable-

gas permeable polymer film. The degree of quenching of the excited complex is related to the partial pressure of O_2 in contact with the film.

According to Bacon, the film serves as an effective barrier to potential interferents such as gases (other than oxygen) and various solutions. The gases and solutions of concern in Bacon were related to those that would potentially damage the sensor dye or produce a false reading. Of particular concern were surfactants, strong oxidants, a reducing agent, a strong acid, and a strong base. See page 2782, column 1. The problem of interferents was solved by providing the sensor in a film wherein the potential interferents including quenchers, solutions, or solvents do not penetrate the polymer, so as to avoid affecting the sensor concentration, degrading the sensor, or distorting the response.

The Bacon reference is silent, however, with respect to utility as an indicator of the presence or absence of oxidative reactions catalyzed by at least one enzyme by direct or indirect contact with a solution containing the enzyme. A fair reading of Bacon teaches that a complex sample, as is the case in which one or more enzymes is present, is likely to contain potential interferents. However, the success of the sensor of Bacon depends on the polymer in which the sensor is contained serving as an effective barrier to potential interferents. "In order for luminescence quenching to be a viable analytical method, potential interferent quenches ***must be excluded*** from the sensor while rapid penetration and equilibration of the quencher to which the sensor must respond were allowed." (emphasis added). See page 2781, column 1, sixth full paragraph. Bacon does establish that contact with gaseous halothane, cyclopropane, CO_2, and nitrous oxide did not affect performance of the sensor. However, these are a mere fraction of the interferents possible, especially in a sample containing enzymes, and which may also contain cells, growth media, and a multiplicity of other potential interferents.

The Bacon reference, for all that it discloses, fails to teach or even suggest the possibility that a sensor, in contact with a sample containing enzymes which catalyze oxidative reactions, would be impermeable to the materials in the sample, including the enzymes. Absent such teaching, it cannot fairly be said that Bacon teaches the method of the invention in which a sample, containing at least compounds which catalyze oxidative reactions, will not interfere with the performance of the sensor. Absent such teaching, it cannot fairly be said that the present invention is obvious in light of the Bacon reference.

In the Parker reference, two generic types of transducers for detecting oxygen concentration are disclosed, both of which are based on fluorescence quenching. One is a hydrophobic transducer and the other is a hydrophilic transducer. In each, a fluorophor is entrapped in a polymer.

The transducer is discussed as ***theoretically*** being amenable to provide a wider class of chemical sensors through coupling to other chemistries. See page 156, first full paragraph. "Similarly, the optical detection of oxygen by fluorescence quenching can be used to detect a wide range of chemicals by coupling the quenching to chemical reactions that either consume or produce oxygen when the desired analyte is present." See page 156, seventh full paragraph. To this end, a theoretical glucose/oxygen transducer is proposed to detect the presence of ***glucose***.

In Parker, the transducers include immobilized glucose oxidase (GO_x) in a PHEMA polymer. See page 159, first full paragraph. "The enzyme was physically entrapped in the polymer by mixing it with the aqueous components of the polymerization mixture prior to polymerization." See page 157, fourth full paragraph. A chemical reaction occurs between the glucose and an oxidized form of glucose oxidase ***in the film*** to form a reduced form of glucose

oxidase, which then consumes oxygen in a chemical reaction. The Parker reference therefore teaches that the analyte (in this case glucose) as well as the enzyme which reacts therewith (glucose oxidase), must be present in the polymer matrix of the transducer and perform a chemical reaction therein, in order to generate the detectable signal. This is in direct contrast to the present invention, where the luminescent compound is present in a polymer film, however, the enzymes are in a gas or solution located ***outside of the film***.

The Parker reference teaches that the reaction to bind oxygen must occur in the film. This teaches away from the present invention because addition of the enzymes to the film would defeat the purpose of the invention, which is to provide a reversible, non-destructive method of measuring oxidative reactions catalyzed by enzymes. Separation of the enzyme from the sample and entrapping it in a polymer with the luminescent compound would prevent the enzymatic activity intended to be measured from occurring. In contrast, the present invention provides a method in which the enzyme may contact the sensor but is not destroyed by such contact. This is what is meant by the limitation that the presence of the sensor is non-destructive to the enzyme. The Parker reference, for all it discloses, does not teach or even suggest such a possibility.

Reconsideration and withdrawal of the rejections of claims 1-12, 14-16, 49-62 and 91-102under 35 U.S.C. §103(a) is appropriate in light of the above and is respectfully requested.

Having responded in full to the outstanding Office Action, it is respectfully submitted that the application, including claims 1-12, 14-16, 49-62, 91, 93-97 and 99-102, is in condition for allowance. An early notice of allowance is respectfully solicited.

The Commissioner is hereby authorized to charge payment of any additional fees associated with this communication or credit any overpayment to Deposit Account No. 08-2461.

If the Examiner has any questions or comments relating to the present application, he or she is respectfully invited to contact Applicant's attorney at the telephone number set forth below.

Respectfully submitted,

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